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ORIGINAL PAPER

Effects of different treatments on seed germination and breaking seed dormancy of *Prosopis koelziana* and *Prosopis Juliflora*

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Abstract: For improving seed germination of *Prosopis koelziana* and *Prosopis juliflora*, different treatments of seeds were conducted, including scarification with sulfuric acid 98% for 10 and 15 min, sandy paper, hot water for 5 and 10 min, potasium nitrate 0.1%, gibberellic acid at 250 $mg \cdot L^{-1}$ and 500 $mg \cdot L^{-1}$ and combinational treatment of scarification with gibberellic acid of 250 $mg \cdot L^{-1}$ and 500 $mg \cdot L^{-1}$. The results show that scarifications with sandy paper and sulfuric acids 98% were the most effective treatments on breaking seed dormancy and seed germination induction. Scarification with sulfuric acid 98% for 15 min was the best treatment. According to the positive effect of scarification and lack of reaction of seeds against KNO_3 and gibberellic acid, the kind of seed dormancy was determined as exogenous.

Keywords: breaking seed dormancy; seed germination; sulfuric acid; *Prosopis koelziana*; *Prosopis juliflora*

Introduction

In recent 20 years, desertification has been recognized as a major environmental problem and is a major focus of United Nations Environment Programme (UNEP). Vegetation is a protector of the soil against water and wind erosion as well as a casualty of soil erosion (Yates et al. 2000; Manzano and Navar 2000). The native plants such as *Prosopis* (Fabaceae) that are adapted to the conditions of arid and semi arid regions are very important for control desertification (Manzano and Navar 2000).

The importance of *Prosopis* as a dryland resource is illustrated in many countries where it is considered a valuable species of the desert ecosystem. Therefore, *Prosopis* is intensively used worldwide in control of desertification of arid and semi arid lands due to its high adaptability for arid conditions. Being a multipurpose tree, *Prosopis* grows very well in dry land agroforestry systems and plays a important role in controlling soil erosion, stabilizing sand dunes, improving soil fertility, reducing soil salinity, providing fuel energy resources, supplying feed and forage for grazing animals, furnishing construction timber and furniture wood, supplementing food for humans, and promoting honey production. The genus *Prosopis* comprises 44 species (Burkart 1976), of which *Prosopis koelziana* and *Prosopis juliflora* are two of the most economically and ecologically important species in arid and semi-arid area. *P. koelziana* is an indicator species of Sahara-Sindy habitats and native species, which is distributed in arid and semi-arid areas of southern Iran (Emtahani and Elmi 2006). *P. koelziana* can grow in soils varying in salinity (EC) from 1 to 3 $dS \cdot m^{-1}$ and can tolerate soil salinity up to 185 $dS \cdot m^{-1}$ (Emtahani and Elmi 2006). This species grew well on soils at pH 7.2 to 8.1 with sodium absorption rate (SAR) from 1 to 7.5. *P. koelziana* has been used to shelter agricultural crops from wind and to reduce the movement of soil and sand dune, and it is also regarded as sources of fuel and animal fodder. This species tolerate hard conditions in salt lands and gypsum regions with sand dunes and growth in area with precipitation of 25–204 mm (Emtahani and Elmi 2006).

P. juliflora is an exotic plant of centre and south of America, which is used in establishment of sand dunes (Ali-El-Keblawy et al. 2005). *P. juliflora* grows in saline soils with electrical conductivity from 8 to 51 $dS \cdot m^{-1}$ (Joshi and Hinglajia 2000) and in sodic soils at pH 10 with exchangeable sodium Percentage (ESP) of 60 in the rooting zone (Singh et al. 1998). However, germination of *P. juliflora* does not occur easily because of its hard seeds. Thus, finding suitable methods for breaking seed dormancy and improving seed germination have been investigated by different researchers (Cox et al. 1945; Vanstone 1978; Khan and Ungar 1985; Derkan and Karssen 1993; Linding and Lara-Carbera 2004; Dehagan et al. 2003; Soyler and Khawar 2006; Soleiman et al. 2008; Fedrico and Mollard 2009). In the present study, different treatments were used for breaking seed dormancy of *P. koelziana* and *P. juliflora,* with an objective to find out the most effective

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method for seed germination and breaking seed dormancy of the two species.

Materials and methods

Seeds of *P. koelziana* and *P. juliflora* were collected from Jiroft and Kahnuj arid regions in southern Iran. A preliminary germination test was performed and very low germination percentage was obtained, indicating that seeds were faced with dormancy. Different treatments for breaking seed dormancy were conducted base on randomized block design with three replicates. Treatments included scarification with sulfuric acid (98%) for 10 and 15 min, sandy paper, hot water for 5 and 10 min, 0.1% KNO_3, gibberellic acid at 250 and 500 mg·L^{-1}. A combinational treatment of sandy paper scarification and gibberellic acid at 250 and 500 mg·L^{-1} was also performed. Seeds were sterilized using 5% sodium hypochlorite solution for 2 min and then washed two times with sterilized water before putting in petridishes. The seeds were placed on top of Whatman paper No. 1 within 10-cm petridishes containing 10-mL distilled water. After this stage, petridishes were transferred to germinator with a temperature of (25±1) ˚C. Counting number of germinating seeds was began from the second day and was done till the end of the experiment (13 days). Finally, germination percentage, germination rate, radicle, coleoptile and seedling length, vigour and mean germination time (MGT) were calculated. Mean germination time was calculated using Ellis and Roberts equation (Ellis and Roberts 1981) to assess the rate of germination (G_R).

$$M_{GT} = \frac{\sum D_i.N_i}{N} \quad (1)$$

$$G_R = \frac{1}{M_{GT}} \quad (2)$$

where, M_{GT} is the mean germination time, N_i the number of seeds germinated on the day i, D_i the days of germination test, N the total number of seeds, and G_R is the germination rate.

Root length and shoot length were measured at the end of experiment. Vigor index was calculated using the following equation:

$$V = G\% \times S \quad (3)$$

where, V is the vigor index, G is the germination percentage, and S is seedling length (Abdul-baki and Anderson 1973). MSTATC program (Microcomputer Program for the Design) was used to analyze the data and Duncan's test at 5% level was used to compare the means.

Results

The results of ANOVA (Table 1) indicated that there were significant differences (at 1% level) between two species in view point of understudy germination characteristics. The different treatments resulted in significant differences among germination properties (Table 1). In addition, interactions of different treatments on species lead to meaningful differences among germination properties except to vigor index (Table 1).

Table 1. Effects of treatments for breaking seed dormancy on germination characteristics of *P. juliflora* and *P. koelziana*

Vigor index	MGT (day)	Seedling length (cm)	Coleoptile length (cm)	Radicle length (cm)	Germination Rate	Germination Percentage (%)	Degree of freedom	Resources
11071.68	0.837	3.581	1.967	0.435	0.003	19.289	2	Replication
3665927.08**	20.135**	346.156**	122.155**	57.046**	0.046**	18637.25**	1	Species
354928.61**	7.547**	43.607**	8.899**	13.92**	0.047**	1403.002**	10	Treatments
34153.52^{ns}	5.464**	19.163**	9.048**	7.69**	0.017**	230.035**	10	Species×Treatments
24356.68	0.895	3.889	1.327	1.245	0.003	81.695	42	Error

Notes: ** significant different as 1% ; *significant different at 5% ; ^{ns} not significantly different.

The effects of all treatments, except KNO_3 on increasing of seed germination percentage of *P. juliflora* were significant (Fig 1). For *P. koelziana*, treatments with KNO_3, gibberellic acid and hot water for 10 min did not show positive effect in germination improvement while scarification with sulfuric acid, sandy paper and combinational treatments of scarification with sandy paper and gibberellic acid at 250 mg·L^{-1} increased percentage of seed germination. Although combinational treatments of scarification with sandy paper and gibberellic acid at 500 mg·L^{-1} and using hot water for 5 min increased seed germination percentage, this increase was not significant. The increase of seed germination percentage was not observed when gibberellic acid (250 and 500 mg·L^{-1}) and KNO_3 0.1% were applied (Fig. 1).

The seed germination rates of *P. juliflora* increased significantly when sulfuric acid was used for 10 and 15 min,(Fig.2); however, the germination rate was decreased when gibberellic acid at 250 mg·L^{-1} and 500 mg·L^{-1} and KNO_3 0.1% were used. Scarification with hot water and combinational treatments of sandy paper and gibberellic acid at 250 mg·L^{-1} and 500 mg·L^{-1} increased seed germination rate, but this increase was not significant. For *P. koelziana*, the seed germination rate was increased significantly for treatments of sulfuric acid, sandy paper and combinational treatments of scarification with sandy paper and gibberellic acid at 250 and 500 mg·L^{-1} (Fig. 2).

Mean germination time of *P. juliflora* decreased due to application of inductional treatments except to the treatments of gibberellic acid and KNO_3. In seeds of *P. koelziana*, all treatments, except to scarification with hot water and KNO_3, caused decrease of mean germination time. A significant decrease in mean germination time was observed for treatments of sulfuric acid, sandy paper, gibberellic acid at 250 mg L^{-1} and combinational treatments of sandy paper- gibberellic acid at 250 and 500 mg·L^{-1}, but

the effect of gibberellic acid at 500 mg·L^{-1} on mean germination time was not significant (Fig. 3). Study of seed vigor showed that scarification with sulfuric acid, sandy paper and combinational treatments of sandy paper- gibberellic acid at 250 and 500 mg L^{-1} resulted in significant vigor increase of both species, while the effect of other treatments on the seed vigor was not significant (Fig. 4). *P. juliflora* had higher vigor than *P. koelziana* (Fig. 5).

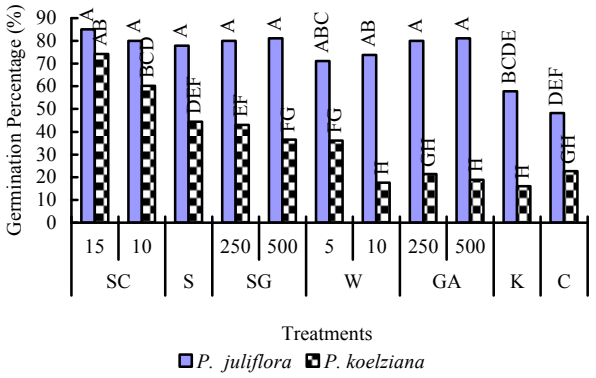

Fig. 1 Effects of treatments for breaking seed dormancy on seed germination percentage of *P. juliflora* and *P. koelziana* (SC is Sulfuric acid for 10 and 15 min, S is Scarific; SG is scarific+Gibberellic at 250 and 500 mg L^{-1}, W is hot water for 5 and 10 min, GA is Gibberellic acid at 250 and 500 mg L^{-1}, K is KNO_3 and C is control)

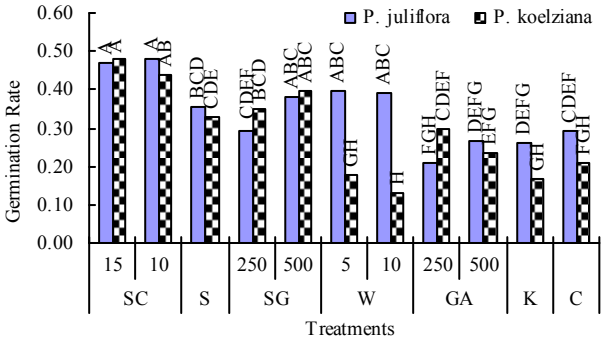

Fig. 2 Effects of treatments for breaking seed dormancy on seed germination rate of *P. juliflora* and *P. koelziana* (SC is Sulfuric acid, S is Scarific; SG is scarific+Gibberellic, W is hot water, GA is Gibberellic acid, K is KNO_3 and C is control)

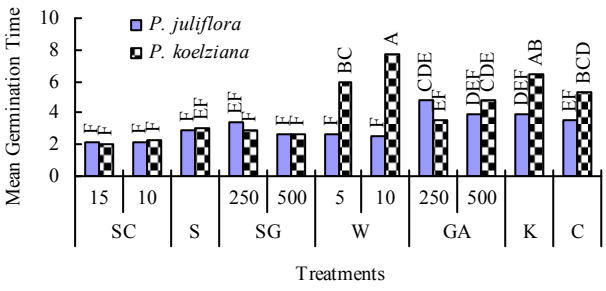

Fig. 3 Effects of treatments for breaking seed dormancy on mean seed germination time of *P. juliflora* and *P. koelziana* (SC is Sulfuric acid, S is Scarific; SG is scarific+Gibberellic, W is hot water, GA is Gibberellic acid, K is KNO_3 and C is control)

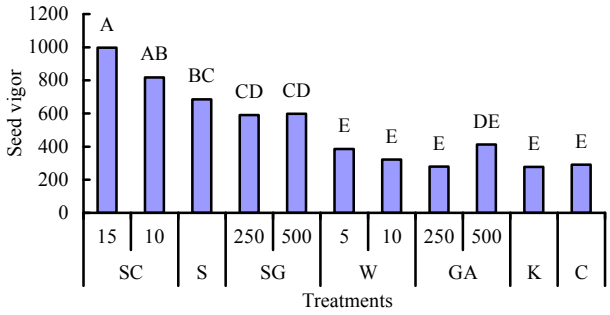

Fig. 4 Effects of treatments for breaking seed dormancy on seed vigor of *P. juliflora* and *P. koelziana* (SC is Sulfuric acid, S is Scarific; SG is scarific+Gibberellic, W is hot water, GA is Gibberellic acid, K is KNO_3 and C is control)

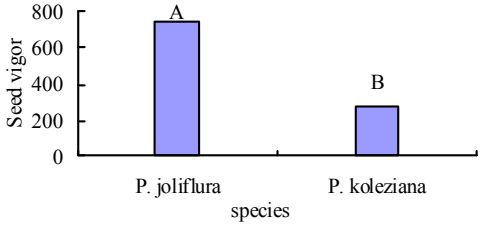

Fig. 5 Seed vigor of *P. juliflora* (A) and *P. koelziana* (B)

Discussion

Germination of a seed depends on the potential of embryo growth or potentials of growth preventor (Koorneef et al. 2002). These potentials depend particularly on seed structure that surrounded the embryo (endosperm, pricarp, glumes). Other factors like hormones and environmental factors also affect embryo growth (Benech et al. 1998; Mares 2005). According to the obtained results, scarification treatments with sandy paper and sulfuric acid (98%) were effective, which caused dormancy breaking and seed germination induction of *P. juliflora* and *P. koelziana*. Our study show that seeds treated with sulfuric acid within 15 min increased germination percentage from 48.21% to 85.01% for *P. juliflora* and from 22.7% to 74.26% for *P. koelziana*. Compared to *P. koelziana, P. juliflora* had a higher germination rate, but its response against sulfuric acid was weaker for germination percentage increment. Sulfuric acid can soften the hard coat of seed and creates a chap on the coat; as a result, seed treatment with sulfuric acid can improve seed germination. Similar results were reported in previous studies for the species of *Dialium guianeese* (McDonald and Omoruyi 2003), *Caparis spona* (Suleiman et al. 2008), *Lupinus diffuses* Nutt (Dehagan et al. 2003), *Parika biglobosa* (Aliero 2004) and *Crotalaria pumila* (Linding and Lara-Carbera 2004). Among all the treatments, after sulfuric acid, scarification with sandy paper showed the highest seed germination rate in addition to vigour of seed. This finding is in agreement with results of Uzen et al. (2004) and Soyler and Khawar (2006). Vanstone (1978) believed that scarification of *Tilia* seeds was necessary to obtain maximum germination. Cox et al. (1945) stated that removal of seed coat could

lead to decrease of preventor's level which in turn results in germination improvement.

For *P. koelziana*, seed germination rate was decreased when seeds were put in hot water for 10 min. This result demonstrated that above mentioned treatment had the destructive effect on embryo. Rincon-Rosales et al. (2003) stated that seeds soaking in hot water cause seed germination induction but increasing duration of seed contact with hot water leads to decline of seed germination percentage. Using chemical materials like gibberellic acid and potasium nitrates had no huge effect on seed germination induction of *P. julifora* and *P. koelziana.* Khan and Ungar (1985) believed that vegetative hormones can break embryo dormancy and neutralize preventation role of Abscissas acid (ABA) directly or indirectly. Derkan and Karssen (1993) have attributed to seed reaction against potasium nitrate to seed sensitivity. According to this fact that mechanical scarification of seed coat resulted in increment of seed germination percentage and rate, also according to elimination of *P. koelziana* and *P. Juliflora* seed dormancy, it could be concluded that dormancy of mentioned species seeds is exogenous. This kind of dormancy happens when factors like water and gas are not permitted to enter the seed, so imbibitions are not occurred and consequently, resulting in decreasing seed germination (Bewley 1997). Occurrence of this kind of dormancy is because of hard coat. In this state, pressure force resulting from water absorption and radicle growth is not enough to break dormancy of seeds. In addition, impermeability of seeds to water and gas was attributed to physical and biochemical obstacles of the coat (Bewley 1997). On the other hand, existence of preventor materials in seed coat could be considered as the reason of this kind of dormancy (Black and Bewley 2000). Since no response was observed when seeds were subjected to the treatments of potassium nitrate, giberlin and combinational treatment of scarification and giberlin; therefore, it is concluded that *P. koelziana* and *P. juliflora* seed dormancy is not derived from physiological factors such as malformed growth of seed and lack of existence of growth regulators in the seeds. In contrast, this confirms existence of exogenous dormancy in *P. koelziana* and *P. juliflora* seeds. According to the obtained results, it is suggested that seeds are exposed to sulfuric acid of 98% within 15 min, which results in overcoming seed dormancy of *P. koelziana* and *P. juliflora*.

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